Institute Report No. 316

Mutagenic Potential of 1,4-Bis[3-(1-Phenylmethoxymethyl)Imidazolium]Butane Dichloride Hemihydrate in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test

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GENETIC TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

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LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Mutagenic Potential of 1,4-Bis[3-(1-Phenylmethoxymethyl)Imidazolium]Butane Dichloride Hemihydrate in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test (Toxicology Series 196)--Sebastian and Korte

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The mutagenic potential of 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Test. Tester strains TA97, TA98, TA100, TA102, TA1535, TA1537, and TA1538 were exposed to doses ranging from 1.0 mg/plate to 0.00032 mg/plate. The test compound was not mutagenic under conditions of this test.

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ABSTRACT

The mutagenic potential of 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE was assessed by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test. Tester strains TA97, TA98, TA100, TA102, TA1535, TA1537, and TA1538 were exposed to doses ranging from 1.0 mg/plate to 0.00032 mg/plate. The test compound was not mutagenic under conditions of this test.

Key Words: Mutagenicity, Genetic Toxicology, Ames Test, 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE, oxime.

PREFACE

TYPE REPORT: Ames Test GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

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Walter Reed Army Institute of Research
Washington, D.C. 20307-5100

PROJECT/WORK UNIT/APC: 3M162734A875/308/TLEO

GLP STUDY NUMBER: 86002

STUDY DIRECTOR: MAJ Don W. Korte Jr., PhD, MSC

PRINCIPAL INVESTIGATOR: Suzanne E. Sebastian, BA, SPC, USA

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocol, retired SOP's, stability and purity data on the test compound, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL) IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE

INCLUSIVE STUDY DATES: 23 April 1986 - 12 September 1986

OBJECTIVE:

The objective of this study was to determine the mutagenic potential of 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL) IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE (LAIR Code TP65) by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

ACKNOWLEDGMENTS

MAJ John W. Harbell, PhD, MSC; SGT Lillie D. Witcher, BS; and Ms. Joanne Wong provided research assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 86002 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte, Jr 27 OCT 88

DON W. KORTE, Jr, PHD / DATE
MAJ, MSC
Study Director

Suzanne E. Sebastian 24 October 88

SUZANNE E. SEBASTIAN, BA / DATE
SPC, USA
Principal Investigator

Conrad Wheeler 27 Oct 88

CONRAD R. WHEELER, PhD / DATE
DAC
Analytical Chemist

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

1 November 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 86Ø02

1. This is to certify that in relation to LAIR GLP Study 86Ø02, the following inspections were made:

15 April 1986	-	Protocol Review
21 May 1986	-	Plate Incorporation (TP62)
17 March 1987	-	Plate Incorporation (TP64)
2Ø March 1987	-	Plate Counting (TP64)

2. The institute report entitled "Mutagenic Potential of 1,4-Bis[3-(1-Phenylmethoxymethyl) Imidazolium] Butane Dichloride Hemihydrate in the Ames Salmonella/Mammalian Microsome Mutagenicity Test," Toxicology Series 196, was audited on 23 April 1987.

Carolyn M. Lewis

CAROLYN M. LEWIS
Chief, Quality Assurance

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Mutagenic Potential of 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test--Sebastian and Korte

INTRODUCTION

1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE was synthesized for a United States Army Medical Research and Development Command program charged with developing more effective oximes for treatment of nerve agent poisoning. The Ames Test is one of a series of tests in which these compounds will be evaluated to determine their relative potential for further development.

The Ames *Salmonella*/Mammalian Microsome Mutagenicity Test is a short-term screening test that utilizes histidine auxotrophic mutant strains of *Salmonella typhimurium* to detect compounds that are potentially mutagenic in mammals. A mammalian microsomal enzyme system is incorporated in the test to increase sensitivity by simulating *in vivo* metabolic activation of the test compound. The Ames Test is an inexpensive yet highly predictive and reliable test for detecting mutagenic activity and thus carcinogenic potential (1).

This evaluation of 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE utilizes a revision of the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test (2). Two new tester strains, a frame-shift strain (TA97) and a strain carrying an ochre mutation on a multicopy plasmid (TA102), are added to the standard tester set.

Objective of the Study

The objective of this study was to determine the mutagenic potential of 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE (LAIR Code TP65) by using the revised Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

MATERIALS AND METHODS

Test Compound

Chemical Name: 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL) IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE

LAIR Code Number: TP65

Physical State: White crystalline solid

Source: SRI International, Menlo Park, CA

Storage: 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL) IMIDAZOLIUM] BUTANE DICHLORIDE HEMIHYDRATE was received from SRI International, 333 Ravenswood Ave., Menlo Park, CA 94025 and assigned the LAIR Code number TP65. The test compound was stored at room temperature (21°C) until used.

Chemical Properties/Analysis: Data provided by SRI International characterizing the chemical composition and purity of the test material are presented in Appendix A along with confirmatory analysis of the test material performed by the Division of Toxicology, LAIR (Presidio of San Francisco, CA).

Test Solvent

The positive control chemicals were dissolved in grade I dimethyl sulfoxide (lot 113F-0450) obtained from Sigma Chemical Co. (St. Louis, MO). The test chemical was dissolved in glass distilled water. Reagent grade water used in this assay was first passed through a Technic Model 301 Reverse Osmosis Unit (Seattle, WA), then through a Corning MP-1 Mega Pure System glass distillation unit (Corning Glass Works, Corning, NY) (3).

Chemical Preparation

On the day of dosing, 100 mg of the test compound was measured into a sterile vial and dissolved in glass distilled water to achieve a 5% (w/v) solution. Aliquots of this solution were used to dose the test plates.

Test Strains

Salmonella strains TA97, TA98, TA100, TA102, TA1535, TA1537, and TA1538 obtained directly from Dr. Bruce Ames, University of California, Berkeley, were used. These strains were maintained in our laboratory in liquid nitrogen.

Quality control tests were run concurrently with the test substance to establish the validity of their special features and to determine the spontaneous reversion rate. Descriptions of the strains, their genetic markers, and the methods for strain validation are given in the LAIR SOP, OP-STX-1 (4).

Test Format

1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE was evaluated for mutagenic potential according to the revised Ames method (2). A detailed description of the methodology is given in LAIR SOP, OP-STX-1 (4).

Toxicity Tests:

Toxicity tests were conducted to determine a sublethal concentration of the test substance. This toxicity level was found by using minimal glucose agar (MGA) plates, concentrations of 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL) IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE ranging from 1.6 x 10^{-3} mg/plate to 5 mg/plate, and approximately 10^{8} cells of TA100 per plate. Top agar containing trace amounts of histidine and biotin was placed on the plates. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth on the plates was observed. Since the highest dose showed neither a decreased number of macrocolonies (below the spontaneous rate) nor an observable reduction in the density of the background lawn, the highest dose selected for the mutagenicity test was 5.0 mg/plate.

Mutagenicity Test:

The test substance was evaluated over a 1000-fold range of concentrations, decreasing from the minimum toxic level (the maximum or limit dose) by a dilution factor of 5, both with and without 0.5 ml of the S-9 microsome fraction. The S-9 (batch R-315) was purchased from Microbiological Associates Inc. (Bethesda, MD). The optimal titer of this S-9, as determined by Microbiological Associates Inc., was 0.75 mg protein/plate. After all the ingredients were added, the top agar was mixed, then overlaid on MGA plates. These plates contained 2% glucose and Vogel Bonner "E" concentrate (5). Plates were incubated upside down in the dark at 37°C for 48 hours. Plates were prepared in triplicate, and the average revertant counts were recorded. The average number of revertants at each dose level was compared to the average number of spontaneous revertants (negative control). The

spontaneous reversion rate (with and without S-9) was monitored by averaging the counts from two determinations run simultaneously with the test compound. The spontaneous reversion rate was determined by inoculating one set of plates before and one set after the test compound plates so that any change in spontaneous reversion rate during the dosing procedure would be detected. This spontaneous reversion rate was also compared with historical values for this laboratory and those cited in Maron and Ames (2). Sterility and strain verification controls were run concurrently. All reagents, test compounds, and media were checked for sterility by plating samples of each on MGA media and incubating them at 37°C with the test plates. The *Salmonella* strains were verified by a standard battery of tests. The integrity of the different *Salmonella* strains used in the assay was verified by the following standard tests:

- -Lack of growth (inhibition) in the presence of crystal violet which indicates that the prerequisite alteration of the lipopolysaccharide layer (LP) of the cell wall is present.

- -Growth in the presence of ampicillin-impregnated disks which indicates the presence of an ampicillin-resistant R Factor in all strains except TA1535, TA1537, and TA1538.

- -Lack of growth (inhibition) following exposure to ultraviolet light which indicates the absence of the DNA excision-repair mechanism (for all strains except TA102).

Six known mutagens were tested as positive controls to confirm the responsiveness of the strains to the mutation process. Each strain must be tested with at least one positive control but may be tested with several. These compounds, benzo[a]pyrene (lot 79C-05252), 2-aminofluorene (lot 021547), 2-aminoanthracene (lot 020797), mitomycin-C (lot 015F-0655), 4-nitroquinoline-n-oxide (lot 89C-0710) and N-methyl-N'-nitro-N-nitrosoguanidine (lot 127C-0342), were obtained from Sigma Chemical Co. (St. Louis, MO). The test compound and mutagens were handled during this study in accordance with the standards published in NIH Guidelines for the Laboratory Use of Chemical Carcinogens (DHHS Publication No. (NIH) 81-2385, May 1981).

Data Interpretation

According to Brusick (6), a compound is considered mutagenic if a positive dose response (correlated dose response) over three dose concentrations is achieved with at least the highest dose yielding a revertant colony count greater than or equal to twice the spontaneous colony count for the tester strains TA98 and TA100, or three times the spontaneous colony count for strains TA1535, TA1537, and TA1538 (2,4). A strong correlated dose response in strain TA100 without a doubling of the individual colony count may also be considered positive.

Maron and Ames (2) consider a compound mutagenic in tester strains TA97 and TA102 if a correlated dose response over three concentrations is achieved with the highest dose yielding a revertant colony count greater than or equal to twice the spontaneous colony count.

Deviations from the Protocol/SOP

There were no deviations from the protocol or standard operating procedures.

Storage of the Raw Data and Final Report

A copy of the final report, study protocols, raw data, SOPs, and an aliquot of the test compound will be retained in the LAIR archives.

RESULTS

On 16 May 1986, the toxicity of 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE was determined (Table 1). For this experiment all sterility, strain verification and negative controls were normal (Table 1). Exposure of the tester strain (TA100) to the highest dose showed a decrease in the number of macrocolonies and an observable reduction in the density of the background lawn, indicating chemical toxicity. Therefore, the highest dose selected for the mutagenicity test was 1.0 mg/plate. Normal results were obtained for all sterility and strain verification tests during the Ames Test performed on 10-12 September 1986 (Table 2). 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL) IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE did not induce any appreciable increase in the revertant colony counts relative to those of the negative control cultures (Table 3). A tabular presentation of the raw data is included in Appendix B.

TABLE 1: TOXICITY LEVEL DETERMINATION FOR TP65

GLP STUDY NUMBER 86002

TOXICITY DETERMINATION REVERTANT PLATE COUNT (TA100)

CONCENTRATION	MEAN	±1SD	BACKGROUND LAWN*
START RUN NEGATIVE CONTROL	77	7.5	NL
5.0 mg/plate	37	8.0	ST
1.0 mg/plate	63	11.9	NL
0.2 mg/plate	62	4.5	NL
0.04 mg/plate	75	8.3	NL
0.008 mg/plate	84	10.2	NL
0.0016 mg/plate	69	10.2	NL
END RUN NEGATIVE CONTROL	92	10.1	NL

STRAIN VERIFICATION FOR TOXICITY DETERMINATION

	TA100*
HISTIDINE REQUIREMENT	NG
AMPICILLIN RESISTANCE	G
UV	NG
CRYSTAL VIOLET SENSITIVITY	NG
STERILITY CONTROL	NG

STERILITY CONTROL FOR TOXICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG

*NL=Normal Lawn, G=Growth, NG=No Growth, ST=Slight Toxicity

TABLE 2: STRAIN VERIFICATION AND STERILITY TESTING FOR THE MUTAGENICITY DETERMINATION OF TP65

GLP STUDY NUMBER 86002

STRAIN VERIFICATION

	OBSERVATIONS*				
STRAIN	HISTIDINE REQUIREMENT	AMPICILLIN RESISTANCE	UV REPAIR	CRYSTAL VIOLET	STERILITY CONTROL
TA97	NG	G	NG	NG	NG
TA98	NG	G	NG	NG	NG
TA100	NG	G	NG	NG	NG
TA102	NG	G	G	NG	NG
TA1535	NG	NG	NG	NG	NG
TA1537	NG	NG	NG	NG	NG
TA1538	NG	NG	NG	NG	NG

STERILITY CONTROL FOR MUTAGENICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG
S-9	NG

*G = Growth, NG = No Growth

TABLE 3: Mutagenicity Assay for 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL) IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE (TP65)†

COMPOUND*	DOSE	TA97		TA98		TA100		TA102	
				WITHOUT S-9					
NEG CONTROL	0.0 mg	115	±11.7	17	±5.1	113	±10.8	56	±8.5
MITO C	0.5 µg							92	±9.2
MNNG	2.0 µg					1277	±223.9		
NQNO	2.0 µg	535	±15.7						
TP65	1.0 mg	103	±12.1	28	±7.8	97	±11.0	71	±9.0
TP65	0.2 mg	129	±7.6	28	±7.8	129	±11.0	61	±5.7
TP65	0.04 mg	98	±2.5	23	±6.0	96	±7.0	50	±6.7
TP65	0.008 mg	105	±3.2	20	±8.6	112	±3.6	48	±5.5
TP65	0.0016 mg	114	±9.3	12	±2.6	105	±13.9	52	±8.5
TP65	0.00032 mg	122	±8.1	17	±4.7	106	±5.9	55	±5.9
				WITH S-9					
NEG CONTROL	0.0 mg	112	±11.0	37	±5.0	102	±18.2	74	±17.0
2-AA	2.0 µg			1982	±254.4	1945	±231.7		
2-AF	2.0 µg	477	±27.6	780	±67.3	177	±17.6		
BP	2.0 µg	427	±32.5	234	±3.5				
TP65	1.0 mg	125	±15.0	54	±4.7	116	±7.2	112	±6.7
TP65	0.2 mg	113	±21.4	30	±11.9	104	±8.7	84	±6.6
TP65	0.04 mg	111	±7.8	36	±1.5	84	±7.2	72	±8.1
TP65	0.008 mg	106	±10.0	21	±4.2	82	±1.2	70	±8.5
TP65	0.0016 mg	114	±13.3	28	±6.7	110	±11.4	61	±17.0
TP65	0.00032 mg	109	±12.7	32	±1.7	101	±7.8	72	±5.6

†Values represent the mean number of revertants/plate (± standard deviation)
*MITO-C=mitomycin-C, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, NQNO=4-nitroquinoline-n-oxide, AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

TABLE 3 (cont.): Mutagenicity Assay for 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL) IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE (TP65)†

COMPOUND*	DOSE/PLATE	TA1535		TA1537		TA1538	
		WITHOUT S-9					
NEG CONTROL	0.0 mg	28	±7.9	10	±2.7	20	±6.4
MNNG	20.0 µg	2693	±157.4				
TP65	1.0 mg	20	±7.6				
TP65	0.2 mg	24	±2.6	9	±4.5	19	±2.6
TP65	0.04 mg	29	±8.7	7	±1.2	17	±2.6
TP65	0.008 mg	30	±5.8	7	±1.0	18	±1.5
TP65	0.0016 mg	25	±3.1	7	±3.8	19	±4.0
TP65	0.00032 mg	25	±5.7	9	±2.5	10	±0.6
				11	±2.1	10	±3.5
		WITH S-9					
NEG CONTROL	0.0 mg	30	±6.7	20	±5.5	28	±4.0
2-AA	2.0 µg			105	±0.0	431	±29.7
2-AF	2.0 µg					395	±392.6
BP	2.0 µg			122	±3.5	112	±20.1
TP65	1.0 mg	27	±4.9	15	±0.6	25	±4.2
TP65	0.2 mg	30	±7.2	23	±7.6	29	±10.1
TP65	0.04 mg	16	±5.9	9	±2.6	21	±3.1
TP65	0.008 mg	22	±6.0	9	±2.6	23	±5.0
TP65	0.0016 mg	22	±8.5	7	±1.0	23	±7.6
TP65	0.00032 mg	29	±3.5	13	±6.2	23	±4.9

†Values represent the mean number of revertants/plate (± standard deviation)
*MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, NQNO=4-nitroquinoline-n-oxide, AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene.

DISCUSSION

Certain test criteria must be satisfied before an Ames Test can be considered a valid assessment of a compound's mutagenic potential. First, the special features of the Ames strains must be verified. These features include demonstration of ampicillin resistance, alterations in the LP layer, and deficiency in DNA excision-repair (except TA102). Second, the *Salmonella* strains must be susceptible to mutation by known mutagens. Third, the optimal concentration of the test compound must be determined by treating TA100 with a broad range of doses and observing the potential toxic effects on formation of macrocolonies and microcolonies. If these tests are performed and expected data are obtained, then the results of an Ames Test can be considered valid.

After validation of bacterial strains and selection of optimal sublethal doses, 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL) IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE was evaluated in the Ames Test. Criteria for a positive response include both a correlated dose response over three dose concentrations, and a revertant colony count at least two times (TA97, TA98, TA100, TA102) (1,6) or three times (TA1535, TA1537, TA1538) (2,4) the spontaneous revertant colony count. 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE did not induce the requisite dose-response relationship or the increase in revertant colony counts necessary for a positive response. Thus, the results of this test indicate that 1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE is not mutagenic when evaluated in the Ames Test.

CONCLUSION

1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE was evaluated for mutagenic potential in the Ames Test, in both the presence and the absence of metabolic activation, and did not induce a positive mutagenic response under conditions of this study.

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APPENDICES

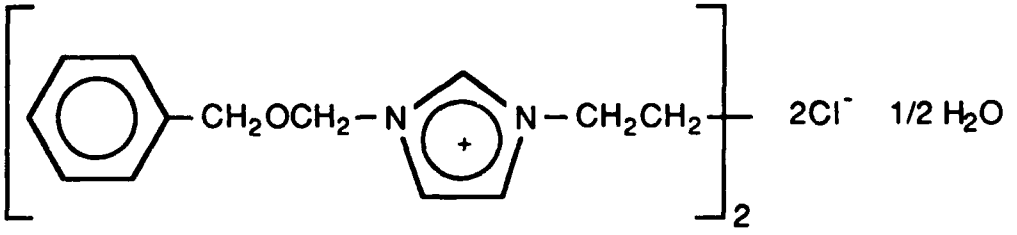
APPENDIX A: Chemical Data

Chemical Name: 1,4-Bis[3-(1-phenylmethoxymethyl)imidazolium] butane dichloride hemihydrate

SRI Reference Number: 6868-32

LAIR Code: TP65

Chemical Structure:



Molecular Formula: $C_{26}H_{32}N_4O_2Cl_2 \cdot 1/2\ H_2O$

Molecular Weight: 512.5

Physical State: White crystalline solid

Analytical Data:

NMR (300 MHz, D_2O): δ 1.81 (s, 4 H, $CH_2C\underline{H}_2C\underline{H}_2CH_2$), 4.19 (s,4 H, $N\text{-}C\underline{H}_2CH_2CH_2C\underline{H}_2\text{-}N$), 4.73 (s, 4 H, phenyl-$C\underline{H}_2$-O), 5.69 (s, 4 H, O-$C\underline{H}_2$-N), 7.36 (m, 10 H, phenyl), 7.46 (s, 2 H, O-CH_2-N--CH--C$\underline{H}$-N), 7.60 (s, 2 H, O-CH_2-N--C$\underline{H}$--CH-N).* The NMR spectrum obtained upon receipt of the compound corresponded closely to the spectrum provided by the source (obtained in DMSO). Any discrepancies were due to the difference in solvents as well as the higher field strength and greater resolution of the NMR used to analyze the compound in our lab. No peaks other than those attributable to the compound were observed in the NMR spectrum.

Stability:

NMR data demonstrate that the compound is stable in water (D_2O) for at least 8 days.†

Source: Clifford D. Bedford
SRI International
Physical Sciences Division
Menlo Park, CA

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*Wheeler CR. Toxicity Testing and Antidotes for Chemical Warfare Agents. Laboratory Notebook #85-12-024, p 9. Letterman Army Institute of Research, Presidio of San Francisco, CA.

†*Ibid.* p 1.

APPENDIX B: Individual Plate Scores

1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE (TP65)

TOXICITY DETERMINATION WITH TA100

DOSE/PLATE	5.0 mg	1.0 mg	0.2 mg	0.04 mg
PLATE 1	29	58	57	68
PLATE 2	45	77	62	72
PLATE 3	37	55	66	84
background lawn	ST*	NL	NL	NL

DOSE/PLATE	0.008 mg	0.0016 mg	NEG START	NEG END
PLATE 1	72	76	81	90
PLATE 2	88	73	81	103
PLATE 3	91	57	68	83
background lawn	NL	NL	NL	NL

* NL=Normal Lawn, ST=Slight Toxicity

APPENDIX B (cont.): Individual Plate Scores

1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE (TP65)

NEGATIVE CONTROL DATA

COMPOUND	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
				WITHOUT S-9				
NEG CONTROL (START RUN)	0.0 mg	135	18	97	60	33	10	28
		111	25	119	66	38	6	21
		101	21	111	63	29	9	22
NEG CONTROL (END RUN)	0.0 mg							
		119	14	124	47	18	12	22
		108	13	104	52	19	14	10
		113	12	122	46	30	10	14
				WITH S-9				
NEG CONTROL (START RUN)	0.0 mg	110	31	82	88	20	26	22
		92	33	87	97	29	13	29
		112	37	89	75	25	13	29
NEG CONTROL (END RUN)	0.0 mg							
		121	39	127	67	31	23	30
		112	39	112	72	34	23	34
		123	45	114	47	39	19	26

APPENDIX B (cont.): Individual Plate Scores

1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE (TP65)

POSITIVE CONTROL DATA

COMPOUND*	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
BP	2.0 µg	462	230	-			120	129
		398	234	-			120	118
		420	237	-			126	90
MITO C	0.5 µg				102			
					90			
					84			
AA	2.0 µg		2205	1680			105	452
			2037	2111			105	410
			1705	2043			-	-
NQNO	2.0 µg	532						
		552						
		521						
MNNG	2.0 µg			1506				
				1260				
				1065				
MNNG	20.0 µg					2514		
						2810		
						2755		
AF	2.0 µg	488	812	157				171
		498	826	190				848
		446	703	184				165

*AA=2-aminoanthracene, AF=2-aminofluorene, BP=benzo[a]pyrene, MITO-C=mitomycin C, MNNG=N-methyl-N'-nitro-N-nitrosoguanidine, NQNO=4-nitroquinoline-n-oxide

APPENDIX B (cont.): Individual Plate Scores

1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE (TP65)

MUTAGENICITY DATA WITHOUT S-9

COMPOUND	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
TP65	1.0 mg	96	22	108	62	28	13	21
		96	26	86	80	18	4	16
		117	37	97	72	13	9	20
TP65	0.2 mg	122	19	138	66	22	8	14
		127	34	133	55	27	8	19
		137	30	117	63	23	6	18
TP65	0.04 mg	101	22	89	46	33	7	16
		98	17	103	58	35	6	19
		96	29	96	47	19	8	18
TP65	0.008 mg	107	29	116	48	23	10	17
		101	12	109	42	33	3	17
		106	18	111	53	33	9	24
TP65	0.0016 mg	117	13	117	44	28	7	9
		104	14	109	61	24	9	10
		122	9	90	51	22	12	10
TP65	0.00032 mg	128	12	104	51	19	10	7
		126	19	113	53	30	13	10
		113	21	102	62	27	9	14

APPENDIX B (cont.): Individual Plate Scores

1,4-BIS[3-(1-PHENYLMETHOXYMETHYL)IMIDAZOLIUM]BUTANE DICHLORIDE HEMIHYDRATE (TP65)

MUTAGENICITY DATA WITH S-9

COMPOUND	DOSE/PLATE	TA97	TA98	TA100	TA102	TA1535	TA1537	TA1538
TP65	1.0 mg	125	49	110	108	23	14	28
		140	56	124	120	-	15	22
		110	58	114	109	30	15	-
TP65	0.2 mg	108	22	94	91	34	15	31
		136	44	106	83	22	25	18
		94	25	111	78	35	30	38
TP65	0.04 mg	120	37	89	68	20	11	20
		109	34	88	66	9	6	24
		105	36	76	81	18	10	18
TP65	0.008 mg	106	16	81	80	21	10	23
		116	22	83	64	16	11	18
		96	24	83	67	28	6	28
TP65	0.0016 mg	111	30	101	78	23	8	15
		103	34	123	62	30	7	30
		129	21	107	44	13	6	25
TP65	0.00032 mg	124	34	92	73	26	20	26
		101	31	106	66	29	8	17
		103	31	105	77	33	11	25

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